antibiotics which target only a small number of bacterial processes. However, with the discovery of riboswitches, we are developing new ways to fight bacterial infections which make use of their own natural metabolic pathways, essentially causing bacteria to destroy themselves. Riboswitches are found in non-coding regions of messenger RNAs and these RNA elements bind to ligands to control the expression of nearby genes. The glucosamine-6-phosphate (glmS) riboswitch is unique in that upon binding its ligand, glucosamine-6-phosphate (GlcN6P), it undergoes self-cleavage and is therefore also a catalytic RNA. The cleavage event targets the RNA for subsequent degradation, thereby repressing further gene expression. To study the glmS riboswitch, initial experiments were performed to determine the mechanism followed upon binding of the natural ligand. Since then, analogs of the natural ligand have been obtained and are being tested for their catalytic capabilities through kinetic analyses and rate constant calculations. Once successful candidates have been determined, these non-natural ligands will be introduced into live bacterial cultures, hopefully disrupting normal cell metabolism and reproduction. If successful, these analogs could be used as novel antibiotics, offering a more specific mode of targeting a wide variety of bacterial species.

1371-Pos

Folding of the Thiamine Pyrophosphate (TPP) Riboswitch

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TPP riboswitches regulate the expression of thiamine-synthesis (*thi*) genes through a variety of mechanisms, all of which involve binding of TPP to a structured aptamer formed in the untranslated region (UTR) of a *thi* mRNA. We used a high-resolution, single-molecule optical trapping assay to characterize mechanically the folding of the TPP riboswitch aptamer located in the 3'UTR of the *thiC* gene of *Arabidopsis thaliana*. Each RNA molecule, containing either the complete aptamer sequence or a portion thereof, was transcribed *in vitro,* annealed to DNA handles via single-stranded overhangs, and placed in a ''dumbbell'' experimental geometry[1]. By applying tension to the ends of the RNA molecule under equilibrium conditions and measuring the corresponding extensions, we observed transitions among several well-defined folding states, which we discuss in the context of secondary and tertiary structures formed by the aptamer[2]. One low-force state of the full aptamer, corresponding to the formation of structural elements located near the three-helix junction, was abolished by mutating a single nucleotide believed to participate in specific tertiary contacts within the junction[2,3]. We observed that the mutant aptamer does not bind TPP or other substrates (thiamine, thiamine monophosphate), and that the wild-type aptamer only binds substrates concomitant with entry into the fully-folded state. We also studied the energetics of substrate binding under non-equilibrium conditions by rapidly increasing or decreasing the extension of the aptamer and measuring the hysteresis in force. The number of phosphates on the substrate modulated the amount of work required to induce substrate unbinding, the height and location of the energy barrier to substrate unbinding, and the amount of RNA released.

1. Greenleaf WJ, et al (2005). PRL 95, 208102.
2. Thore S, et al (2006). Science 312, 1208-1211.
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1372-Pos

Structure and Function of a Potential Mammalian Riboswitch

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Riboswitches, found in untranslated regions of mRNAs, bind to specific cellular metabolites and undergo a conformational change which modifies expression of a nearby coding region of the mRNA. This coding region is involved in the synthesis of the same metabolite, thereby providing an efficient feedback mechanism of genetic control. To date, various riboswitches have been described to effectively control genetic expression in bacterial cells, but none have been discovered in mammals. We are investigating the structure and function of a potential mammalian riboswitch, thought to control polyamine biosynthesis. Polyamines surround cellular DNA to stabilize the DNA negative charge. To validate this small RNA as a new riboswitch, we are using in-line probing to verify specific metabolite binding and subsequent conformational change. Additionally, to verify the ability of the potential riboswitch to control gene expression, in vivo studies are being performed using a reporter gene system. Successful results from both of these investigations will determine whether this small RNA is a true riboswitch. Further investigations will include determination of its tertiary structure. It is known that cancer cells require a higher concentration of polyamine due to their increased replication rate. Thus, a combination of structural and functional studies of this RNA may prove useful in the development of novel cancer therapies.

1373-Pos

Dynamics of the Catalytic Pocket of a Diels-Alder Ribozyme

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The Diels-Alder ribozyme, an in vitro-evolved highly abundant ribonucleic acid enzyme, accelerates the formation of carbon-carbon bonds between a diene and a dienophile in a [4+2] cycloaddition reaction, a reaction with broad application in biochemistry and organic chemistry.
We have examined the ribozyme in the unbound form in solid and liquid phase by means of Molecular Dynamics simulations of 1 microsecond total simulation time. Our simulations confirm highly dynamic state of the catalytic pocket as observed by recent NMR spectroscopy studies.
However, the preformed catalytic pocket architecture, suggested previously based on X-ray investigations, exists only under certain conditions. Simulations of the crystal state show that at the temperature of 100K the catalytic pocket remains in its starting conformation. Yet, at the transitional temperature of 250K a collapse of the catalytic pocket occurs, and the ribozyme adopts an ezymatically inactive closed conformation of the pocket.
Simulations in solution performed at 300K at different magnesium ions concentration reveal that the stabilization of the catalytic pocket depends on high amounts of Mg-ions. At higher Mg^{2+} concentrations the cations are more likely to bind to the backbone of those residues that bridge the opposite strands of the pocket, which leads to stabilization of the enzymatically active open conformation. Simulations with artificial constraints confirm and quantify the effect of backbone stabilization on a catalytically active state. At too low Mg-ion concentrations, catalytically inactive states with a collapsed catalytic pocket dominate. In these conformations the ribozyme is not able to host any reactant. The catalytically active state with an open pocket is a metastable state that can only be accessed and is only sufficiently stabilized at a high enough magnesium concentration, explaining the experimentally found full catalytic activity dependence on the Mg-ions concentration.

1374-Pos

Structural Probing of the T Box Antiterminator-tRNA Complex

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Structural changes in a unique RNA-RNA binding interaction were probed using 2-aminopurine. The 5′-untranslated leader region (5′-UTR) of the T box family of genes folds into a structure that selectively recognizes a specific tRNA through two unique base-pairing events. The first involves base pairing between the anticodon of cognate tRNA and a tri-nucleotide sequence (specifier sequence) in the specifier loop of stem 1 in the 5′-UTR. The second base pairing event involves the non-aminoacylated tRNA acceptor end base pairing with the first four nucleotides at the 5′-end of a bulge in a highly conserved antiterminator element. In the absence of the stabilization of the uncharged tRNA acceptor end base pairing to the antiterminator element, the more thermodynamically stable terminator element forms and transcription terminates. In this manner, the leader region specifically recognizes cognate tRNA and responds structurally to the charging ratio of the tRNA to regulate transcription, thus making the T box mechanism an example of a riboswitch. Interestingly, the predicted thermodynamic stabilization provided by the four base pairs between the tRNA acceptor end and the antiterminator is not sufficient to overcome the predicted stability difference between the antiterminator and the terminator elements. Consequently, additional structural factors likely play a role in stabilizing the resulting complex. The structural changes induced in both the antiterminator element and the tRNA were investigated using a model system to determine what additional factors, beyond base pairing, contribute to stabilization of the resulting tRNA-antiterminator complex. Fluorescence monitoring of the base analog 2-aminopurine at select positions throughout a model complex indicated that binding results in an induced-fit and a highly stacked environment at the binding interface. These structural features contribute to the overall stabilization of the complex beyond the four base pairs.

1375-Pos

Nanosecond Motions of the Substrate-Recognition Duplex in a Group I Intron Assessed by Site-Directed Spin Labeling

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The *Tetrahymena* group I intron recognizes its oligonucleotide substrate in a two-step process. First, a substrate recognition duplex, called the P1 duplex,